

Supplementary Tables and Figures

The contraceptive medroxyprogesterone acetate, unlike norethisterone, directly increases R5 HIV-1 infection in human cervical explant tissue at physiologically relevant concentrations

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Supplementary Tables

Supplementary Table S1: Hormone value ranges for different phases of the menstrual cycle taken from NHLS.

	Follicle stimulating hormone(IU/L)	Luteinising hormone (IU/L)	Oestradiol (E2) (pmol/L)	Progesterone (nmol/L)
Follicular phase:	3.5 - 12.5	2.4 - 12.6	45 – 854	0.2 - 2.8
Ovulation phase:	4.7 - 21.5	14.0 - 95.6	151 - 1461	0.4 - 38.1
Luteal phase:	1.7 - 7.7	1.0 - 11.4	82 – 1251	5.8 - 75.9
Postmenopausal:	25.8 - 134.8	7.7 - 58.5	<18 – 505	<0.2 - 0.4

Supplementary Table S2: Donor Table

Donor	HSV 1&2 IgG	HSV 1 IgG	HSV 2 IgG	HSV IgM	LH (IU/L)	FSH (IU/L)	E2 (pmol/L)	P4 (pmol/L)	Age	Stage of menstrual cycle	Indication for surgery	Figure used
1	positive				5.5	8.7	83.7	9.1	51	Follicular	Ultrasound showed hemipelvic mass Abnormal uterine bleeding (Menorrhagia), failed medical treatment, Anaemia	Figs. 1a, 2a
2		positive	positive	negative	10.4	6.1	230.4	1.6	44	Follicular	Fibroid uterus, Menorrhagia, prolonged menses, Dysmenorrhea	Figs. 1a, 2a, 2c
3		positive	negative	negative	9	6	2629	0.9	48	Irregular prolonged bleeding	Fibroid uterus, Menorrhagia, Anaemia	Figs. 1a, 2a
4		positive	negative	negative	3	5.9	84.2	0.5	44	Follicular	Ovarian mass (likely benign)	Figs. 1a, 2a, 2c
5	positive				3.2	4.1	462.6	40.5	53	Perimenopausal - Luteal	Multi-fibroid uterus	Figs. 1a, 1d, 2a, 2b, 5a-h
6	positive				6.9	14.7	395.2	16.5	41	Early luteal	Unknown	Figs. 1a, 2a, 5a-h, 6
7	positive				Nd	Nd	Nd	Nd	42	Irregular	Multi-fibroid uterus	Figs. 1a, 1b, 1d, 2a, 2b, 5a-h
8	positive				1.6	4.3	238	7.9	46	Luteal	Multi-fibroid uterus	Figs. 1a, 1d, 2a, 2b, 3c, 5a-h
9	positive				0.9	3.2	128	6.6	47	Luteal	Unknown	Figs. 1a, 1d, 2a, 2b, 5a-h
10		positive	negative	negative	11	11.3	179	2.4	49	Follicular	Abdominal distension, abdominal pain Urinary incontinence, uterine prolapse, Grade II cystocele, Grade I uterine prolapse & rectocele	Figs. 1a, 1b, 1c, 2a, 5a-h, 5i-l
11		positive	negative	positive	13.1	10.5	231	8.5	48	Ovulatory	Perimenopausal - Irregular	Figs. 1a, 1b, 1e, 5a-l
12	positive				21.6	43.2	<19	<0.7	53	Multi-fibroid uterus		Figs. 1a, 1b, 1c, 1d, 2b

Donor	HSV 1&2 IgG	HSV 1 IgG	HSV 2 IgG	HSV IgM	LH (IU/L)	FSH (IU/L)	E2 (pmol/L)	P4 (pmol/L)	Age	Stage of menstrual cycle	Indication for surgery	Figure used
13		positive	negative	positive	7.2	7.7	286.4	0.3	49	Follicular Phase	Adenomyomatous uterus, Nabothian cyst on cervix	Figs. 1a, 1b, 1c, 5i-l
14		positive	negative	negative	5.1	2.8	390	7.2	45	Luteal phase	Pelvic organ prolapse	Figs. 1a, 1b, 1c, 1d, 2b, 5i-l
15		positive	negative	positive	7.8	6.4	329	19.1	46	Luteal Phase	Pelvic organ prolapse III procedentia	Figs. 1a, 1b, 1c, 5i-l
16	Nd				29.6	61.3	120	2.9	46	Perimenopausal - Irregular	Fibroid uterus	Figs. 1a, 1b, 1e, 3c, 5a-h, 5i-l
17	positive				1.3	5.2	33	<0.7	47	Perimenopausal - Irregular	Uterine prolapse	Figs. 1a, 3c, 5a-h
18	positive				0.6	3.7	?	2.6	46	Perimenopausal - Irregular	Multi-fibroid uterus	Figs. 1a, 3c
19	Nd				3.8	6.3	270	6.6	Nd	Luteal		Fig. 1a
20	positive				0.8	4.1	52	Nd	42	Irregular	Unknown	Fig. 5a-h
21	positive				Nd	Nd	Nd	Nd	44	Irregular	Uterine polyp Fibroid uterus, history of dysmenorrhea & menorrhagia	Fig. 1b
22	positive				4.3	3.9	19	2.9	54	Follicular		Figs. 1e, 5i-l
23	positive				Nd	Nd	Nd	Nd	45	Irregular prolonged bleeding	Multi-fibroid uterus	Fig. 1e
24		positive	positive	negative	12.1	23.2	46	<0.1	39	Irregular	Heavy menstrual bleeding	Fig. 1e
25		positive	negative	negative	4.4	4.8	163.5	1	37	Follicular	Fibroid uterus, heavy menstrual bleeding	Fig. 2c
26		positive	negative	negative	35.7	63.8	39.3	0.5	46	Perimenopausal - Irregular	Uterine prolapse	Fig. 2c

Donor	HSV 1&2 IgG	HSV 1 IgG	HSV 2 IgG	HSV IgM	LH (IU/L)	FSH (IU/L)	E2 (pmol/L)	P4 (pmol/L)	Age	Stage of menstrual cycle	Indication for surgery	Figure used
27		negative	negative	negative	5.6	6.3	476.3	0.9	38	Follicular	Menorrhagia for two years	Fig. 2c
28		positive	positive	negative	0.6	3.3	212.4	0.5	41	Late Luteal	Abnormal uterine bleeding, single fibroid	Fig. 2c
29	Nd				Nd	Nd	Nd	Nd	49	Irregular	Multi-fibroid uterus	Figs. 3a, 6
30	positive				1.6	3.7	107	1.1	52	Perimenopausal	Endometrial Hyperplasia	Figs. 3a, 6
31	positive				Nd	Nd	Nd	Nd	44	Irregular	Multi-fibroid uterus	Fig. 4
32	positive				Nd	Nd	Nd	Nd	44	Irregular	Multi-fibroid uterus	Fig. 4
33	positive				Nd	Nd	Nd	Nd	43	Irregular	Multi-fibroid uterus, symptomatic anaemia	Fig. 4
34		positive	negative	negative	4.4	8.8	313	<0.1	48	Irregular prolonged bleeding	Menorrhagia - fibroid uterus	Figs. 4, 5a-h, 48h FACS
35		positive	positive	negative	1.9	4.6	95	0.3	50	Follicular	Heavy menstrual bleeding due to multi-fibroid uterus	Figs. 4, 5a-h, 48h FACS
36					<0.1	0.1	<19	0.6	40	Follicular	Abnormal uterine bleeding, failed medical management	Figs. 4, 5a-h
37		positive	negative	negative	12	14	74	1.4	43	Irregular	Multi-fibroid uterus	Figs. 4, 5a-h
38		positive	negative	negative	22.6	12.2	922	0.3	44	Follicular	Multi-fibroid uterus	Figs. 4, 5a-h, 48h FACS
39		positive	negative	negative	1.2	0.8	227	<0.2	40	Irregular	Vesicovaginal fistula repair	Figs. 4, 5a-h
40		positive	negative	negative	<0.1	0.1	<19	0.9	44	Irregular	Multi-fibroid uterus, abnormal uterine bleeding	Figs. 4, 5a-h, 7

Donor	HSV 1&2 IgG	HSV 1 IgG	HSV 2 IgG	HSV IgM	Hormone levels				Age	Stage of menstrual cycle	Indication for surgery	Figure used
					LH (IU/L)	FSH (IU/L)	E2 (pmol/L)	P4 (pmol/L)				
41		positive	negative	negative	0.7	5.3	27	2.8	45	Follicular	Multi-fibroid uterus	Figs. 4, 5a-h, 7
42		positive	negative	negative	6.8	7.9	163	<0.2	45	Follicular	Abnormal uterine bleeding	Figs. 4, 5a-h, 7
43		positive	positive	negative	5.5	12.2	131	2	49	Follicular	Multi-fibroid uterus	Figs. 4, 5a-h, 7
44		positive	negative	negative	40.9	49	349	0.2	51	Perimenopausal - Irregular	Uterine fibroid	Figs. 4, 5a-h, 7
45		positive	positive	negative	14.3	11.1	313	8.2	51	Perimenopausal - Irregular	Abnormal uterine bleeding	Figs. 4, 5a-h, 7
46		positive	negative	negative	2.1	1.1	30	<0.1	51	Irregular prolonged bleeding	Heavy prolonged menstrual bleeding due to multi-fibroid uterus	Figs. 4, 5a-h, 7
47		Positive	negative	negative	12.5	17.9	196	3.6	40	Irregular prolonged bleeding	Abnormal uterine bleeding, Ovarian cyst	Figs. 4, 5a-h, 7
48		Positive	negative	negative	37.9	63.9	60	<0.1	53	Irregular prolonged bleeding	Fibroid uterus, pressure symptoms, abnormal uterine bleeding, failed medical treatment	Figs. 4, 5a-h
49		Positive	positive	negative	2.8	4.2	164	4	43	Luteal	Heavy menstrual bleeding	Figs. 4, 5a-h
50	positive				4.6	4	162	Nd	51	Irregular prolonged bleeding	Abnormal uterine bleeding	Figs. 5a-h, 6
51	positive				1.4	3.6	Nd	7.6	41	Luteal	Multi-fibroid uterus	Figs. 5a-h, 6
52	positive				9.3	17.5	99.9	<0.7	46	Follicular	Abnormal uterine bleeding with multi-fibroid uterus	Fig. 6
53	Nd				1.1	3.2	<19	2.6	42	Irregular	Fibroids causing urinary symptoms	Fig. 6

Donor	HSV 1&2 IgG	HSV 1 IgG	HSV 2 IgG	HSV IgM	Hormone levels				Age	Stage of menstrual cycle	Indication for surgery	Figure used
					LH (IU/L)	FSH (IU/L)	E2 (pmol/L)	P4 (pmol/L)				
54	positive				4.3	8.9	100	0.8	36	Irregular prolonged bleeding	Abnormal uterine bleeding	Fig. 6
55	positive				0.5	2.4	132	5.7	34	Irregular	Multi-fibroid uterus	Fig. 6
56		Positive	negative	negative	7.2	6.9	413	0.6	44	Irregular	Fibroid uterus, heavy menses, pelvic pain	Fig. 6
57		Positive	negative	negative	6.3	6.1	537.1	0.3	48	Follicular	Fibroid uterus, backache, urinary frequency, abnormal uterine bleeding	Fig. 6
58		Positive	positive	negative	4.5	5.6	89	0.5	48	Perimenopausal- Irregular	Right sided pelvic pain	Fig. 6
59		Positive	negative	negative	7.7	5.8	105.3	0.9	42	Follicular Phase	Fibroid uterus, abnormal uterine bleeding	Fig. 6

Tygerberg Hospital simultaneously test for HSV1&2 IgG, while Groote Schuur Hospital and Somerset Hospital performs individual tests for HSV1 IgG, HSV2 IgG and HSV IgM

Nd - Not Determined

Irregular as indicated by clerking sheet or lack of identifiable phase

Supplementary Table S3A: Repeated measures 2-way ANOVA for 100 nM MPA treated

Ectocervical explants infected with HIV-1 BaLRenilla

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	38.18	<0.0001	****	Yes
Treatment	1.335	0.1003	ns	No
Interaction: Time x Treatment	0.5175	0.113	ns	No

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle - 100 nM MPA					
0	0	-0.4939 to 0.4939	No	ns	>0.9999
3	-0.2621	-0.756 to 0.2318	No	ns	0.5959
5	-0.3862	-0.8801 to 0.1076	No	ns	0.196
7	-0.5581	-1.052 to -0.06419	Yes	*	0.0194
10	-0.6665	-1.16 to -0.1726	Yes	**	0.0033

Statistics performed on n = 19 due to missing time point (day 3) for one donor

Supplementary Table S3B: Repeated measures 2-way ANOVA for 10 nM MPA treated

Ectocervical explants infected with HIV-1 BaLRenilla

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	39.28	<0.0001	****	Yes
Treatment	0.0396	0.4896	ns	No
Interaction: time x Treatment	0.03516	0.634	ns	No

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle - 10 nM MPA					
0	0	-0.2319 to 0.2319	No	ns	>0.9999
3	-0.002037	-0.2339 to 0.2298	No	ns	>0.9999
5	0.06	-0.1719 to 0.2919 -0.08094 to	No	ns	0.9638
7	0.1509	0.3828	No	ns	0.3588
10	0.115	-0.1169 to 0.3469	No	ns	0.6408

Supplementary Table S3C: Repeated measures 2-way ANOVA for 1 nM MPA treated Ectocervical explants infected with HIV-1 BaLRenilla .

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	50.18	0.0008	***	Yes
Treatment	0.2206	0.5064	ns	No
Interaction: Time x Treatment	0.172	0.6452	ns	No

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle - 1 nM MPA					
0	0	-0.6744 to 0.6744	No	ns	>0.9999
3	0.001333	-0.6731 to 0.6757	No	ns	>0.9999
5	0.2183	-0.4561 to 0.8927	No	ns	0.8926
7	0.2957	-0.3787 to 0.9701	No	ns	0.7115
10	0.4193	-0.2551 to 1.094	No	ns	0.3729

Supplementary Table S3D: Repeated measures 2-way ANOVA for 10 nM MPA treated Endocervical explants infected with HIV-1 BaLRenilla

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	65.43	<0.0001	****	Yes
Treatment	1.633	0.1537	ns	No
Interaction: time x treatment	0.7645	0.1161	ns	No

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle - 10 nM MPA					
0	0	-0.6743 to 0.6743	No	ns	>0.9999
3	-0.295	-0.9693 to 0.3793	No	ns	0.7131
5	-0.365	-1.039 to 0.3093	No	ns	0.5147
7	-0.8383	-1.513 to -0.1641	Yes	*	0.0115
10	-0.7433	-1.418 to -0.06906	Yes	*	0.0271

Supplementary Table S4A: Repeated measures 2-way ANOVA for Ectocervical explants infected with HIV-1BaL-Renilla

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	51.53	<0.0001	****	Yes
Treatment	1.713	0.0861	ns	No
Interaction: Time x Treatment	0.6922	0.007	**	Yes

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Day 3					
Vehicle vs. 100 nM MPA	-0.233	-0.5454 to 0.0795 -0.3091 to	No	ns	0.1814
Vehicle vs. 100 nM NET	0.003333	0.3158	No	ns	0.9996
100 nM MPA vs. 100 nM NET	0.2363	-0.07617 to 0.5488	No	ns	0.173
Day 5					
Vehicle vs. 100 nM MPA	-0.3756	-0.688 to -0.06309 -0.1454 to	Yes	*	0.0146
Vehicle vs. 100 nM NET	0.167	0.4795	No	ns	0.4101
100 nM MPA vs. 100 nM NET	0.5426	0.2301 to 0.8551	Yes	***	0.0003
Day 7					
Vehicle vs. 100 nM MPA	-0.4207	-0.7332 to -0.1083 -0.1054 to	Yes	**	0.0055
Vehicle vs. 100 nM NET	0.207	0.5195	No	ns	0.2574
100 nM MPA vs. 100 nM NET	0.6278	0.3153 to 0.9402	Yes	****	<0.0001
Day 10					
Vehicle vs. 100 nM MPA	-0.4756	-0.788 to -0.1631 -0.01802 to	Yes	**	0.0015
Vehicle vs. 100 nM NET	0.2944	0.6069	No	ns	0.0688
100 nM MPA vs. 100 nM NET	0.77	0.4575 to 1.082	Yes	****	<0.0001

Statistics performed on n = 9 due to missing time point (day 3) for one donor

Supplementary Table S4B: Repeated measures 2-way ANOVA for Endocervical explants infected with HIV-1 Bal-Renilla

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	10.25	0.1639	ns	No
Treatment	1.705	0.3868	ns	No
Interaction: Time x Treatment	1.182	0.4305	ns	No

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Day 3					
Vehicle vs. 100 nM MPA	-1.121	-4.53 to 2.289	No	ns	0.7051
Vehicle vs. 100 nM NET	0.3183	-3.091 to 3.728	No	ns	0.972
100 nM MPA vs. 100 nM NET	1.439	-1.97 to 4.849	No	ns	0.5642
Day 5					
Vehicle vs. 100 nM MPA	-4.228	-7.638 to -0.8189	Yes	*	0.012
Vehicle vs. 100 nM NET	-0.6656	-4.075 to 2.744	No	ns	0.8834
100 nM MPA vs. 100 nM NET	3.563	0.1534 to 6.972	Yes	*	0.0389
Day 7					
Vehicle vs. 100 nM MPA	-2.585	-5.995 to 0.8241	No	ns	0.168
Vehicle vs. 100 nM NET	-2.473	-5.882 to 0.9369	No	ns	0.1943
100 nM MPA vs. 100 nM NET	0.1128	-3.297 to 3.522	No	ns	0.9964
Day 10					
Vehicle vs. 100 nM MPA	-2.807	-6.217 to 0.6022	No	ns	0.1243
Vehicle vs. 100 nM NET	-1.64	-5.05 to 1.769	No	ns	0.4771
100 nM MPA vs. 100 nM NET	1.167	-2.242 to 4.576	No	ns	0.6849

Supplementary Table S4C: Repeated measures 2-way ANOVA for Ectocervical explants infected with HIV-1 pNL4-3.

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	28.88	0.0171	*	Yes
Treatment	2.196	0.1011	ns	No
Interaction: time x Treatment	1.264	0.0426	*	Yes

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Day 3					
Vehicle vs. 100 nM MPA	-0.08111	-0.6746 to 0.5124	No	ns	0.9395
Vehicle vs. 100 nM NET	0.2374	-0.3561 to 0.8309	No	ns	0.5911
100 nM MPA vs. 100 nM NET	0.3185	-0.275 to 0.912	No	ns	0.3937
Day 5					
Vehicle vs. 100 nM MPA	-0.1167	-0.9611 to 0.7277	No	ns	0.934
Vehicle vs. 100 nM NET	0.7583	-0.08607 to 1.603	No	ns	0.0828
100 nM MPA vs. 100 nM NET	0.875	0.0306 to 1.719	Yes	*	0.0416
Day 7					
Vehicle vs. 100 nM MPA	-0.1067	-0.7002 to 0.4868	No	ns	0.8978
Vehicle vs. 100 nM NET	0.8568	0.2633 to 1.45	Yes	**	0.0035
100 nM MPA vs. 100 nM NET	0.9635	0.37 to 1.557	Yes	**	0.0011
Day 10					
Vehicle vs. 100 nM MPA	-0.1633	-0.7568 to 0.4302	No	ns	0.7777
Vehicle vs. 100 nM NET	0.8466	0.2531 to 1.44	Yes	**	0.0039
100 nM MPA vs. 100 nM NET	1.01	0.4165 to 1.603	Yes	***	0.0006

Supplementary Table S5: Repeated measures 2-way ANOVA for 100 nM MPA +- RU486

treated ectocervical explants infected with HIV-1 BaLRenilla

Source of Variation	% of total variation	P value	P value summary	Significant?
Time	70.12	<0.0001	****	Yes
Treatment	3.975	0.3188	ns	No
Interaction: Time x Treatment	1.311	0.1862	ns	No

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Day 3					
Vehicle vs. 100 nM MPA	-0.3938	-0.7715 to -0.01604	Yes	*	0.0383
Vehicle vs. RU486	-0.04375	-0.4215 to 0.334	No	ns	0.9893
Vehicle vs. RU486/MPA	-0.03125	-0.409 to 0.3465	No	ns	0.996
100 nM MPA vs. RU486	0.35	-0.02771 to 0.7277	No	ns	0.0777
100 nM MPA vs. RU486/MPA	0.3625	-0.01521 to 0.7402	No	ns	0.0639
RU486 vs. RU486/MPA	0.0125	-0.3652 to 0.3902	No	ns	0.9997
Day 5					
Vehicle vs. 100 nM MPA	-0.4	-0.7777 to -0.02229	Yes	*	0.0345
Vehicle vs. RU486	0.0375	-0.3402 to 0.4152	No	ns	0.9932
Vehicle vs. RU486/MPA	0.09375	-0.284 to 0.4715	No	ns	0.9083
100 nM MPA vs. RU486	0.4375	0.05979 to 0.8152	Yes	*	0.0179
100 nM MPA vs. RU486/MPA	0.4938	0.116 to 0.8715	Yes	**	0.0062
RU486 vs. RU486/MPA	0.05625	-0.3215 to 0.434	No	ns	0.9778
Day 7					
Vehicle vs. 100 nM MPA	-0.3688	-0.7465 to 0.008959	No	ns	0.0578
Vehicle vs. RU486	0.175	-0.2027 to 0.5527	No	ns	0.6012
Vehicle vs. RU486/MPA	0.225	-0.1527 to 0.6027	No	ns	0.389
100 nM MPA vs. RU486	0.5438	0.166 to 0.9215	Yes	**	0.0023
100 nM MPA vs. RU486/MPA	0.5938	0.216 to 0.9715	Yes	***	0.0008
RU486 vs. RU486/MPA	0.05	-0.3277 to 0.4277	No	ns	0.9842
Day 10					
Vehicle vs. 100 nM MPA	-0.3375	-0.7152 to 0.04021	No	ns	0.0939
Vehicle vs. RU486	0.1688	-0.209 to 0.5465	No	ns	0.6288
Vehicle vs. RU486/MPA	0.2875	-0.09021 to 0.6652	No	ns	0.1891
100 nM MPA vs. RU486	0.5063	0.1285 to 0.884	Yes	**	0.0049
100 nM MPA vs. RU486/MPA	0.625	0.2473 to 1.003	Yes	***	0.0004
RU486 vs. RU486/MPA	0.1188	-0.259 to 0.4965	No	ns	0.8318

Supplementary Table S6. Mean and standard deviations of observed protein concentrations determined by Luminex or ELISA

Soluble mediator	Dose	Treatment	Mean pg/mL	Standard Deviation
Eaotaxin⁽¹⁾	100 nM	Vehicle	39.81	25
		MPA	45.16	31.72
		NET	34.43	25.07
	10 nM	Vehicle	32.71	22.83
		MPA	30.22	20.09
		Vehicle	4414	5913
IL-1RA⁽¹⁾	100 nM	MPA	3090	1800
		NET	4265	4479
	10 nM	Vehicle	1619	2573
		MPA	620.2	626.5
IL6⁽¹⁾	100 nM	Vehicle	2038	3361
		MPA	2234	3624
		NET	6206	12802
	10 nM	Vehicle	515.6	1065
		MPA	1832	1986
IL-6⁽²⁾	100 nM	Vehicle	2622	1699
		MPA	2480	1574
		NET	2503	1717
IL8⁽¹⁾	100 nM	Vehicle	8187	4415
		MPA	7621	4372
		NET	7287	4623
	10 nM	Vehicle	7836	3899
		MPA	7899	4697
		Vehicle	8755	4049
IL-8⁽²⁾	100 nM	MPA	8269	3786
		NET	7733	4800
		Vehicle	2870	2104
MCP-1⁽¹⁾	100 nM	MPA	2639	2077
		NET	2617	2291
		Vehicle	2935	2077
	10 nM	MPA	3043	2545
		Vehicle	107.8	51.95
RANTES⁽¹⁾	100 nM	MPA	95.62	49.66
		NET	107.9	61.11
		Vehicle	46.43	41.18
	10 nM	MPA	53.96	48.61
		Vehicle	7123	6310
SLPI⁽¹⁾	100 nM	MPA	5800	5642
		NET	9248	7693
		Vehicle	15345	17106
	10 nM	MPA	18093	18154
SLPI⁽²⁾	100 nM	Vehicle	8301	6807
		MPA	7600	5967
		NET	6363	8314

Mean concentration (pg/mL) and standard deviation of the soluble mediator as measured by
 (1) Luminex or (2) ELISA.

Supplementary Table S7: % viability of ungated population following 48 hr and 7 days post treatment

48 hr		Vehicle		MPA		NET	
		%viable	% dead	%viable	% dead	%viable	% dead
Donor 34		97.6	2.36	97.7	2.31	96.3	3.72
Donor 35		94.8	5.22	98.7	1.29	99.4	0.64
Donor 38		73	27	65.1	34.9	72.2	27.8
Mean		88.47	11.53	87.17	12.83	89.30	10.72
SD		13.47	13.48	19.12	19.12	14.89	14.87
Day 7		Vehicle		MPA		NET	
		%viable	% dead	%viable	% dead	%viable	% dead
Donor 40		97.1	2.88	98.6	1.36	98.7	1.35
Donor 41		99.1	0.92	98	1.96	94.8	5.24
Donor 42		99.9	0.057	99.4	0.61	99.8	0.2
Donor 43		99.6	0.41	99.6	0.44	99.6	0.4
Donor 44		99.1	0.87	99.2	0.79	98.1	1.9
Donor 45		99.5	0.54	94.3	5.75	99.8	0.22
Donor 46		97.7	2.3	94.3	5.68	99.3	0.7
Donor 47		99.7	0.3	99.1	0.87	99.4	0.64
Mean		98.96	1.03	97.81	2.18	98.69	1.33
SD		1.02	1.01	2.22	2.23	1.68	1.68

Supplementary Table S8A. Frequencies of T cells and monocytes expressing CD69 or CCR5 in ectocervical explants stimulated with 100 nM MPA or NET versus control for 48 hr

Immune cell phenotype	Vehicle	100 nM MPA	100 nM NET
CD3+ % total	42.53 (14.5)	36.8 (8.764)	26.73 (11.230)
CD3+CCR5+	97.83 (1.084)	97.2 (1.904)	98.63 (0.367)
CD3+CD69+	37.03 (12.15)	40.57 (16.69)	37.67 (15.39)
CD3+CD69+CCR5+	99.3 (0.436)	98.73 (0.819)	98.97 (0.176)
CD4+ % total	24.29 (11.57)	26.88 (14.2)	17.96 (6.794)
CD4+CCR5+	96.53 (1.943)	97 (1.29)	97.93 (0.561)
CD4+CD69+	48.03 (13.48)	50.8 (18.06)	53.4 (14.46)
CD4+CD69+CCR5+	97.87(1.749)	97.7 (1.15)	98.46 (0.841)
CD8+ % total	36.83 (5.63)	35.3 (2.954)	33.2 (4051)
CD8+CCR5+	99.27 (0.41)	99.1 (0.5)	99.33 (0.12)
CD8+CD69+	36.13 (11.13)	39.43 (15.71)	37.76 (16.93)
CD8+CD69+CCR5+	99.76 (0.12)	99.43 (0.47)	99.1 (0.361)
CD14+ % total	56.23 (16.41)	60.9 (22.52)	39.86 (12.53)
CD14+CCR5+	96.16 (0.376)	96.67 (0.318)	92.3 (2.495)
CD14+CD69+	16.64 (6.005)	13.87 (7.981)	11.48 (5.123)
CD14+CD69+CCR5+	99.33 (0.441)	96.33 (2.717)	98.13 (1.065)
CD4+/CD8+ ratio	0.64 (0.311)	0.84 (0.507)	0.504 (0.153)

Average frequency (\pm SEM) indicated for n=3 independent explant donors. Statistical comparisons were carried between conditions out using parametric one-way ANOVA with Tukey's post-test or non-parametric Kruskal-Wallis with Dunn's post-test.

Supplementary Table S8B. Densities of cells expressing CD69 or CCR5 in ectocervical explants stimulated with 100 nM MPA or NET versus control for 48 hr

Immune cell phenotype	Vehicle	100 nM MPA	100 nM NET
CD3+CCR5+ MFI	29112 (8718)	24794 (7624)	25724.33 (5849)
CD3+CD69+MFI	15887.67 (8123)	16028.33 (8174)	16709.67 (8594)
CD3+CD69+CCR5+ (CCR5 MFI)	30711.67 (9163)	27288.66 (8929)	28534.33 (6801)
CD3+CD69+CCR5+ (CD69 MFI)	15884.33 (8126)	16037 (8171)	16709.67 (8594)
CD4+CCR5+ MFI	28819.33 (8880)	26576.33 (8974)	26467 (5604)
CD4+CD69+MFI	17890.67 (8647)	16957.33 (7961)	17637.67 (7995)
CD4+CD69+CCR5+ (CCR5 MFI)	32268.67 (10007)	30073.67 (10502)	31838 (8834)
CD4+CD69+CCR5+ (CD69 MFI)	17937.67 (8616)	16998.33 (7940)	17652 (7989)
CD8+CCR5+ MFI	26738 (7906)	22847 (5789)	24259 (5604)
CD8+CD69+MFI	15400 (8082)	15326.33 (8011)	16342 (8934)
CD8+CD69+CCR5+ (CCR5 MFI)	27791.33 (8434)	25732.67 (7721)	25206.33 (5816)
CD8+CD69+CCR5+ (CD69 MFI)	15400 (8082)	15326.33 (8011)	16343 (8932)
CD14+CCR5+ MFI	61244 (36253)	32539.67 (12817)	31581.33 (10061)
CD14+CD69+MFI	6688.67 (2967)	6243 (2369)	6674.67 (2599)
CD14+CD69+CCR5+ (CCR5 MFI)	94254.67 (53090)	53462 (28496)	42570.67 (10660)
CD14+CD69+CCR5+ (CD69 MFI)	6688.33 (2967)	6243 (2369)	6804.33(2712)

Average median fluorescent intensity (MFI) \pm SEM indicated for n=3 independent explant donors. Statistical comparisons were carried between conditions out using parametric one-way ANOVA with Tukey's post-test or non-parametric Kruskal-Wallis with Dunn's post-test.

Supplementary Table S9A. Frequencies of T cells and monocytes expressing CD69 or CCR5 in ectocervical explants stimulated with 100 nM MPA or NET versus control for 7 days

Immune cell phenotype	Vehicle	100 nM MPA	100 nM NET
CD3+ % total	31.24 (10.28)	38.85 (6.746)	31.91 (10.3)
CD3+CCR5+	77.1 (3.181)	83.48 (2.923)	78.21 (5.402)
CD3+CD69+	25.11 (4.957)	25.8 (4.237)	26.66 (6.348)
CD3+CD69+CCR5+	88.6 (3.337)	91.76 (1.991)	91.19 (1.995)
CD4+ % total	2.804 (1.127)	4.349 (1.743)	5.26 (2.47)
CD4+CCR5+	87.71(4.456)	89.13 (5.041)	90.58 (2.166)
CD4+CD69+	68.94 (6.257)	57.33(4.137)	62.14 (6.939)
CD4+CD69+CCR5+	91.04 (4.894)	92.65(4.99)	91.73 (2.467)
CD8+ % total	22.03 (5.091)	23.91 (3.823)	24.3 (6.617)
CD8+CCR5+	87.26 (2.874)	91.78 (1.924)	88.84 (2.81)
CD8+CD69+	55.2 (3.658)	54.18 (5.047)	59.01 (3.206)
CD8+CD69+CCR5+	88.99 (3.431)	94.16 (2.062)	91.78(2.884)
CD14+ % total	20.62 (6.31)	30.92 (5.45)	19.67 (5.349)
CD14+CCR5+	97.43 (1.064)	98.28 (0.4898)	97.55 (0.9194)
CD14+CD69+	13.74 (2.388)	18.92 (2.761)	15.25 (2.58)
CD14+CD69+CCR5+	99.05 (0.3742)	99.56 (0.1647)	99.49 (0.2649)
CD4/CD8 ratio	0.1556 (0.06186)	0.1944 (0.06786)	0.2175 (0.07601)

Average frequency (\pm SEM) indicated for n=8 independent explant donors. Statistical comparisons were carried between conditions out using parametric one-way ANOVA with Tukey's post-test or non-parametric Kruskal-Wallis with Dunn's post-test. **Bold** indicates fold change significance, as shown in Figure 7.

Supplementary Table S9B. Densities of cells expressing CD69 or CCR5 in ectocervical explants stimulated with 100 nM MPA or NET versus control for 7 days

Immune cell phenotype	Vehicle	100 nM MPA	100 nM NET
CD3+CCR5+ MFI	7114 (452.1)	7376 (468.9)	7778 (674.4)
CD3+CD69+MFI	3572 (370.9)	3639 (418.6)	3726 (340.4)
CD3+CD69+CCR5+ (CCR5 MFI)	9194 (971)	9268 (878.1)	9766 (992.3)
CD3+CD69+CCR5+ (CD69 MFI)	3531 (365.5)	3639 (430.7)	3703 (342.6)
CD4+CCR5+ MFI	8798 (1003)	10190 (1635)	12808 (2053)
CD4+CD69+MFI	3705 (602.5)	3757 (500.3)	4370 (543.6)
CD4+CD69+CCR5+ (CCR5 MFI)	9265(1054)	10635 (1829)	13251 (2097)
CD4+CD69+CCR5+ (CD69 MFI)	3728 (593.6)	3794 (521.5)	437 (584)
CD8+CCR5+ MFI	8076(714.7)	8520 (781.4)	8616 (923.4)
CD8+CD69+MFI	4061 (312.8)	4127 (422.1)	4299 (289.5)
CD8+CD69+CCR5+ (CCR5 MFI)	8679 (876.4)	9057 (944.5)	9142 (1020)
CD8+CD69+CCR5+ (CD69 MFI)	4026 (297.5)	4108 (420.9)	4260 (262.6)
CD14+CCR5+ MFI	12685 (1316)	13583 (1277)	13798 (1203)
CD14+CD69+MFI	2785 (177.8)	2946 (189.3)	2907 (190.3)
CD14+CD69+CCR5+ (CCR5 MFI)	17551 (2094)	20019 (2445)	19944 (1995)
CD14+CD69+CCR5+ (CD69 MFI)	2782 (177.3)	2944 (189.2)	2901 (189.9)

Average median fluorescent intensity (MFI) \pm SEM indicated for n=8 independent explant donors. Statistical comparisons were carried between conditions out using parametric one-way ANOVA with Tukey's post-test or non-parametric Kruskal-Wallis with Dunn's post-test.

Supplementary Table S10: Absolute cell number of CD4 and CD4CCR5+ cells of individual donors

Absolute cell number	CD4+			CD4+CCR5+		
48 hr	Vehicle	100 nM MPA	100 nM NET	Vehicle	100 nM MPA	100 nM NET
Donor 34	926	582	824	897	554	805
Donor 35	666	408	197	664	406	195
Donor 38	993	572	583	923	551	566
	861.7	520.7	534.7	828	503.7	522
Mean (\pmSD)	(172.7)	(97.7)	(316.3)	(142.6)	(84.6)	(307.4)
Day 7						
Donor 40	256	605	252	216	454	135
Donor 41	25	143	67	23	138	65
Donor 42	8	144	15	7	144	14
Donor 43	660	632	973	515	514	806
Donor 44	488	728	757	423	617	682
Donor 45	170	2126	95	165	2078	84
Donor 46	946	5817	2683	924	5676	2643
Donor 47	174	319	165	159	296	155
	340.9	1314	625.9	304	1240	573
Mean (\pmSD)	(330.5)	(1926)	(901.5)	(306.7)	(1898)	(888.6)

Absolute cell number of CD4+ and CD4+CCR5+ cells of individual donors at 48 hr (n=3) and 7 days (n=8) post stimulation as measured by flow cytometry on gated viable CD3+ cells (as shown in gating strategy in figure 3S). Mean (\pm SD) of donors 48 hr (n=3) and 7 days (n=8) post stimulation are also indicated.

Supplementary Table S11: MFI of CD4 cells of individual donors

48 hr	CD4 MFI		
	Vehicle	100 nM MPA	100 nM NET
Donor 34	5640	5359	5750
Donor 35	4481	4986	5169
Donor 38	3518	3208	3693
Mean (\pmSD)	4546.33 (1062.5)	4517.66 (1149.43)	4870.66 (1060.45)
Day 7			
Donor 40	3539	3525	3372
Donor 41	6733	6432	5652
Donor 42	7253	6631	7414
Donor 43	3539	3800	3902
Donor 44	5093	4024	5268
Donor 45	4008	3934	4566
Donor 46	2609	2702	3590
Donor 47	2928	3132	2815
Mean (\pmSD)	4462.75 (1733.78)	4272.5 (1461.48)	4572.37 (1496.09)

Notes: MFI: Median fluorescent Intensity

Supplementary Figures

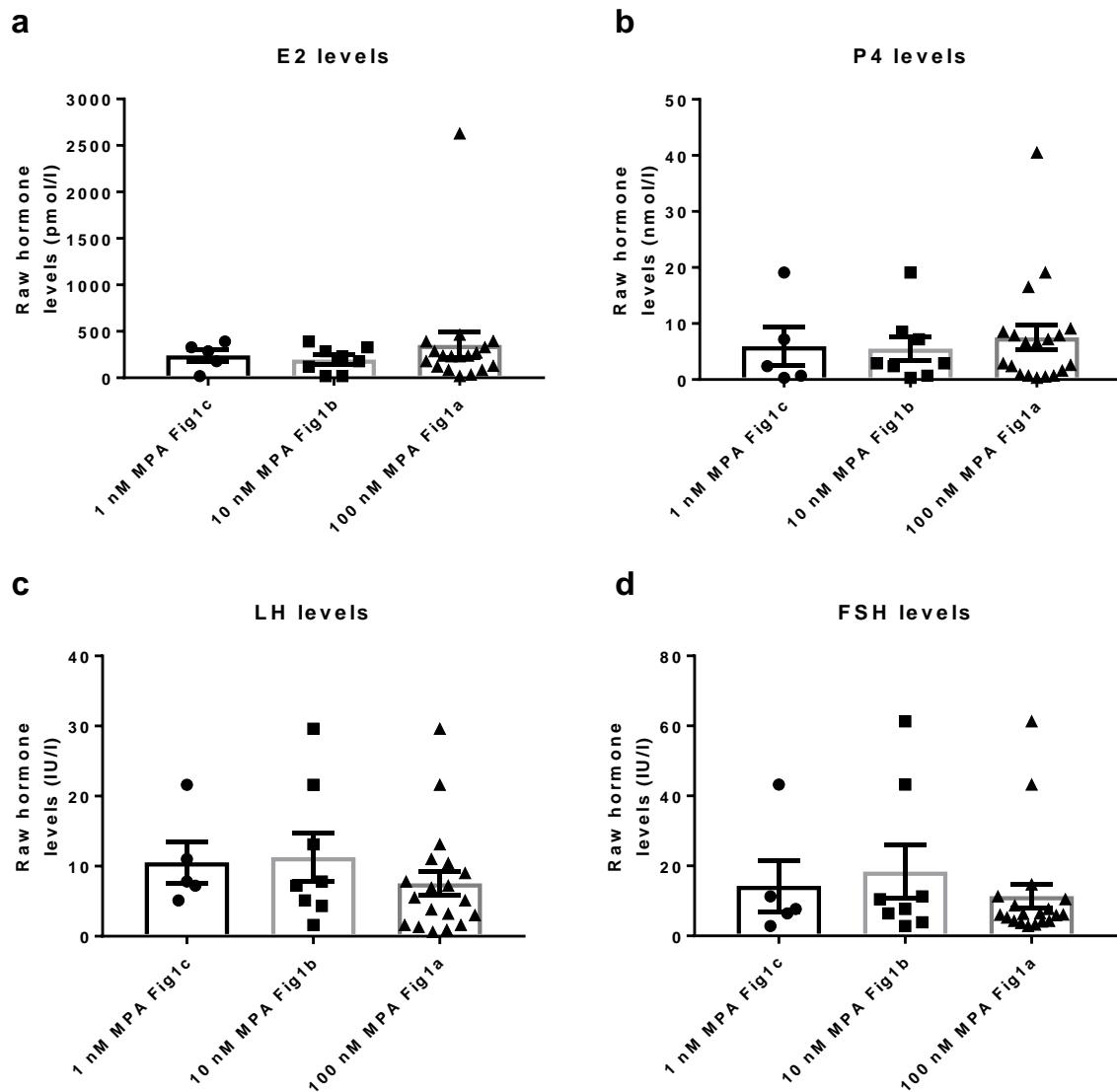


Figure S1: Donor endogenous hormone levels are not significantly different.

Endogenous E2 (a), P4 (b), LH (c) and FSH (d) hormone levels available for the individual donors used in tissue infection experiments from Figure 1 (a-c) were pooled. For two of the donors used in Figure 1 (a-c), the hormone levels are not known and they have been excluded from this graph. Histograms show mean \pm SEM of n = 5 for 1 nM MPA, n = 8 for 10 nM MPA and n = 19 for 100 nM MPA.

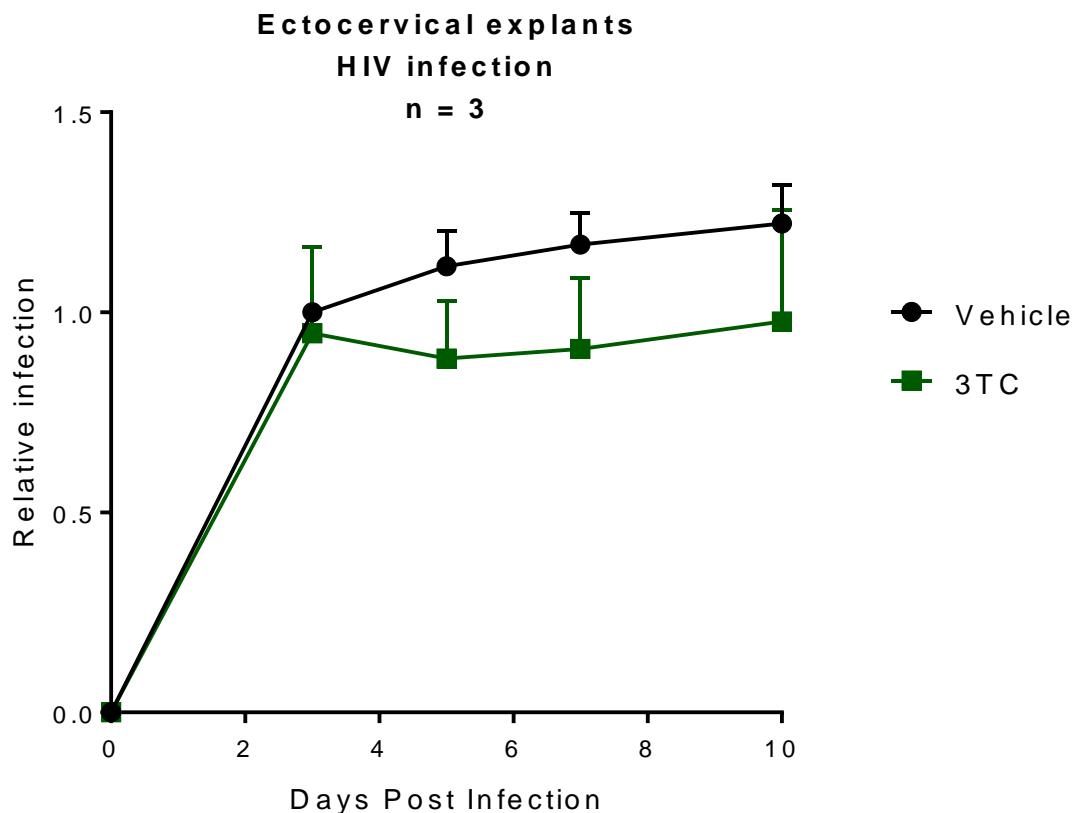


Figure S2: Ectocervical explants are productively infected with HIV-1.

Explants were treated in parallel with vehicle (DMSO (0.1% v/v)) or 5 (n = 1) or 10 μ M (n = 2) 3TC for 1 (n = 2) or 48 hr (n = 1) before adding 1000 (n = 2) or 10000 (n = 1) IU/mL HIV-1 IMCs and incubating for a further 2 hr. Thereafter explants were washed several times with 1 X PBS and added back to fresh media. Half of the supernatants were collected every 2 – 3 days and media were replaced in the presence of 3TC. The variations in time of preincubation with 3TC, [3TC] and infectious units of HIV-1 as indicated did not detectably change the results. Relative infection was determined by p24 ELISA analysis, and normalized by setting the day 3 vehicle to 1. Graphs show cumulative curve of p24 production over time (from days 0, 3, 5, 7 and 10 post HIV infection). Each condition was performed in triplicate and pooled data is shown for three independent experiments with three different donors, with XY plots showing mean \pm SEM.

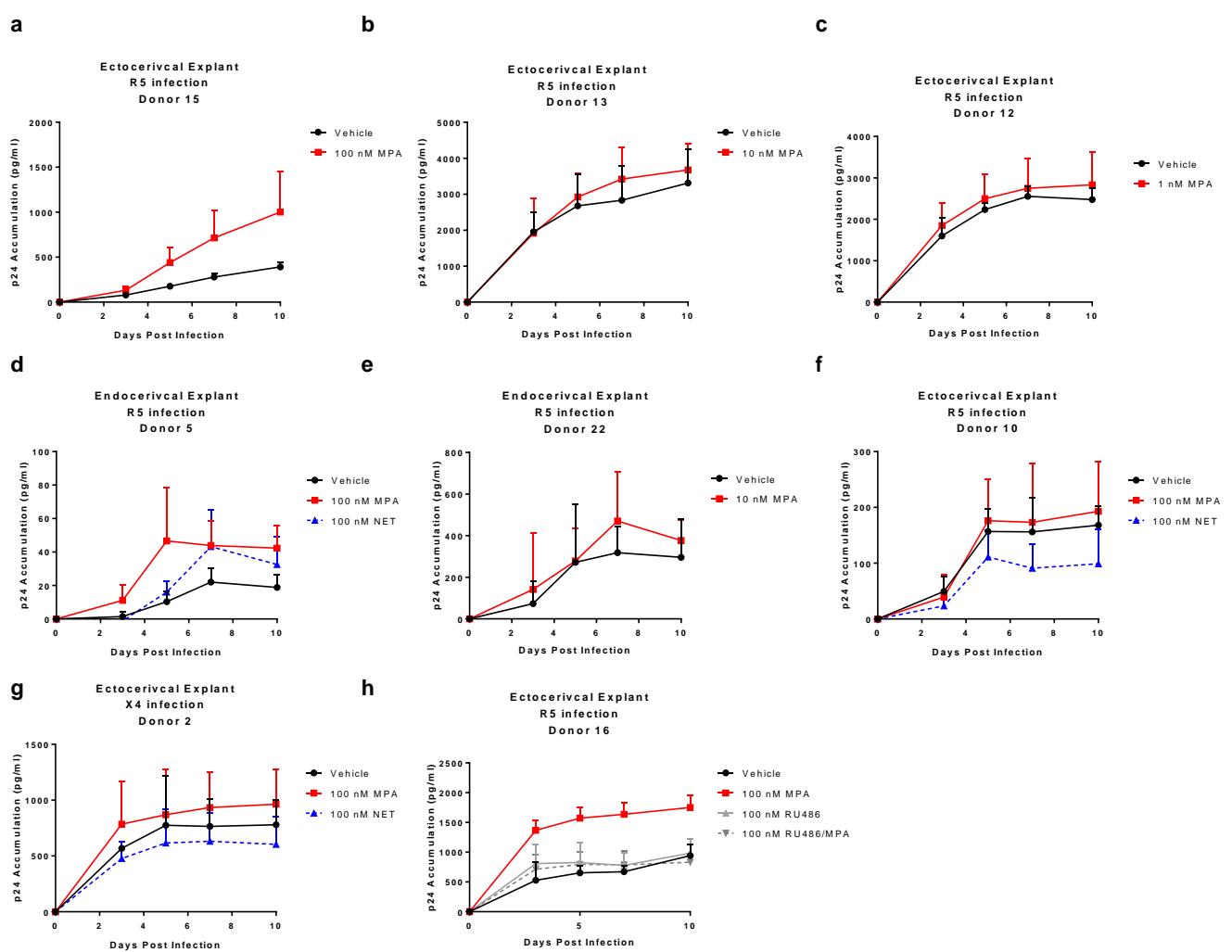
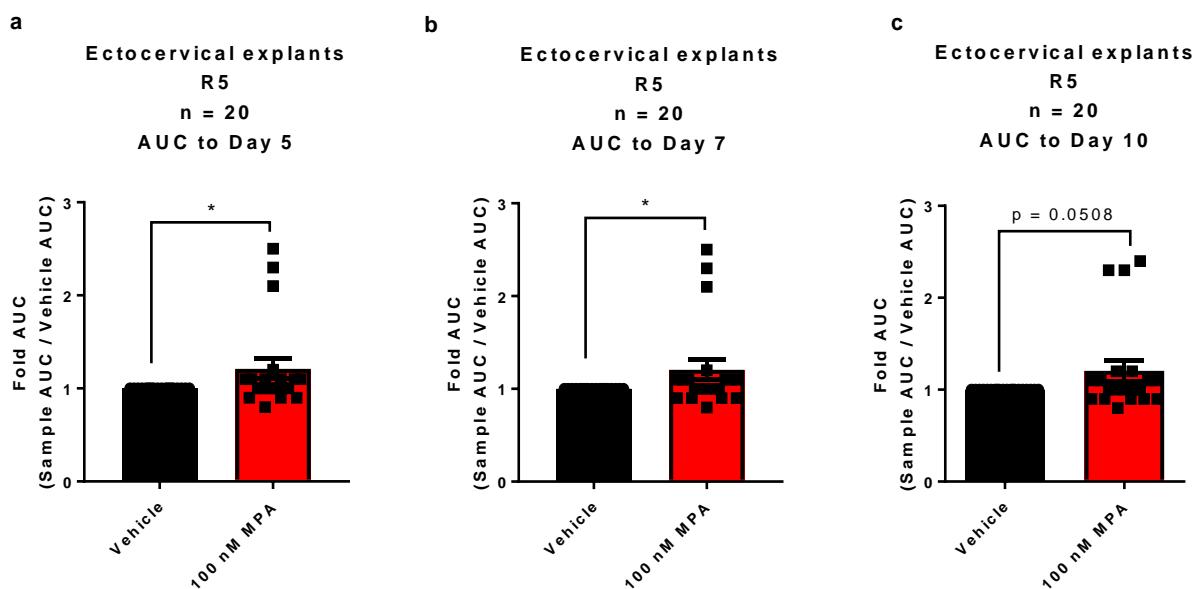


Figure S3: p24 accumulation curves over time for individual representative donor cervical explants. Representative p24 infection data (pg/mL) are shown for individual donor tissue infection experiments from Figures 1 - 3. Explants were pre-treated in parallel for 48 hr in parallel with vehicle or progestins as indicated in Figures 1 - 3 and graphs show p24 accumulation at time points 0, 3, 5, 7 and 10 days post infection. Explants were infected with either HIV-1_{BaL-Renilla} (a,c,d-h) or HIV-1_{PNL4.3} (b). Each condition was performed at least in triplicate. Data is representative of individual donors, with XY plots showing mean + SD.



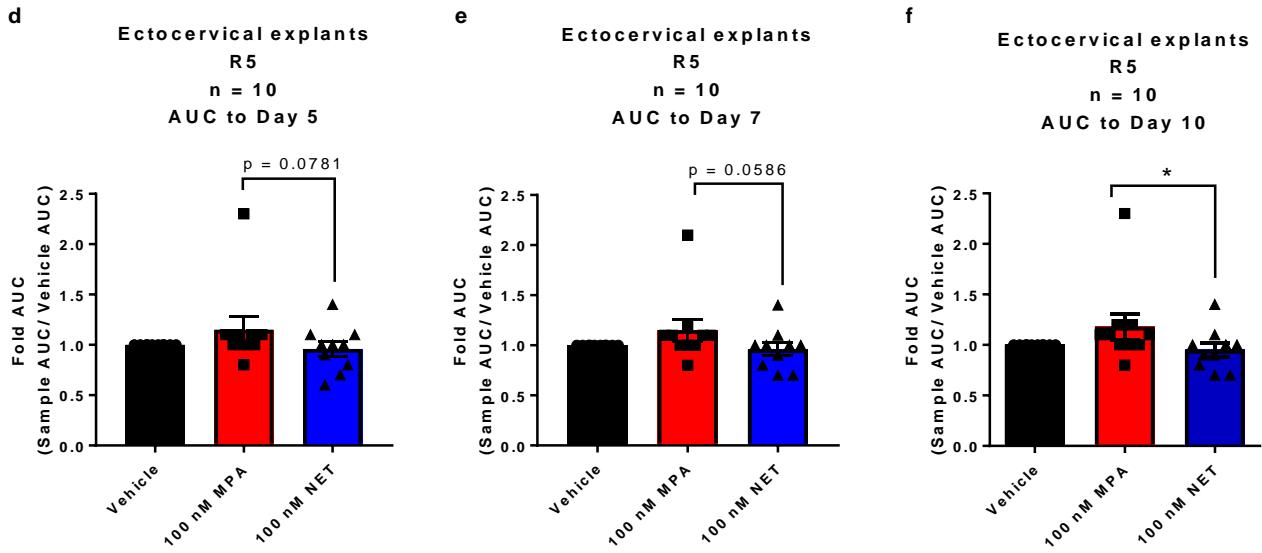


Figure S4: Area under the curve analyses were performed on R5 infected ectocervical explants treated with (a-c) 100 nM MPA or vehicle control as well as R5 infected ectocervical samples treated with (d-f) 100 nM MPA or NET. AUCs were calculated over different time points with (a and d) AUC up to Day 5, (b and e) up to Day 7 and (c and f) up to Day 10 post infection. AUCs were calculated using Graphpad Prism software version 6, with AUCs normalized to the vehicle control set to 1. Histograms are representative of mean \pm SEM. For a-c, non-parametric paired Wilcoxon signed rank tests were performed on each of the data sets with p = 0.00179 for Day 5, p = 0.0258 for Day 7 and p = 0.0508 for Day 10. For (f), a non-parametric Friedman one-way ANOVA was performed with a post-hoc Dunn's test for multiple comparisons with p = 0.0417 for MPA vs NET. For (d) and (e) a non-parametric paired Wilcoxon signed rank tests were performed with p = 0.0781 (d) and p = 0.0586 (e).

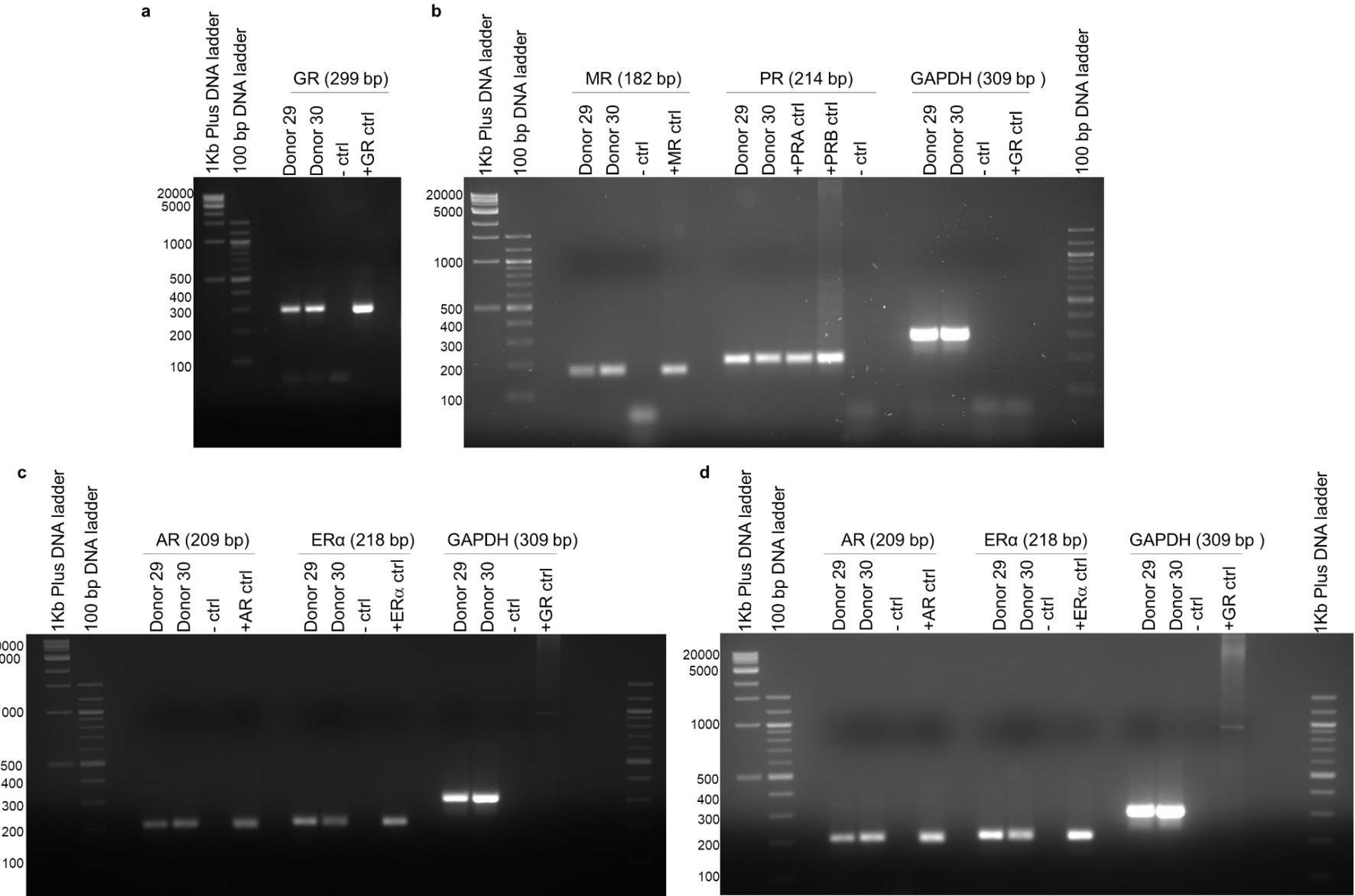


Figure S5. Steroid receptor mRNA levels showing separate gels and exposures used for

Figure 3. Total RNA from two donors was analysed for the different steroid receptor mRNAs by RT-PCR. RT-PCR products were electrophoresis on an agarose gel stained with ethidium bromide and products visualised under UV for steroid receptor mRNA (see methods for details). Separate gels were used for electrophoreses of (a) GR products (1 min exposure) (b) MR and PR and GAPDH products (3 mins exposure) and (c-d) AR and ER α and GAPDH products with two exposures of (c) 1 min and (d) 3 mins. Sizes were determined by comparison to the 1 Kb plus and 100 bp DNA ladders were as indicated. For presentation purposes in Figure 3A, products were cropped out of respective gels and arranged in order of specific products of Donor 29 and 30, -ctrl (H₂O) and + ctrl (positive steroid receptor control plasmid). GR products and MR products were cropped from the gel a, b. The PR products were cropped from gel b and rearranged to fit the presentation style. GAPDH products were cropped from gel c to avoid overexposure of GAPDH and AR and ER α products were cropped from d.

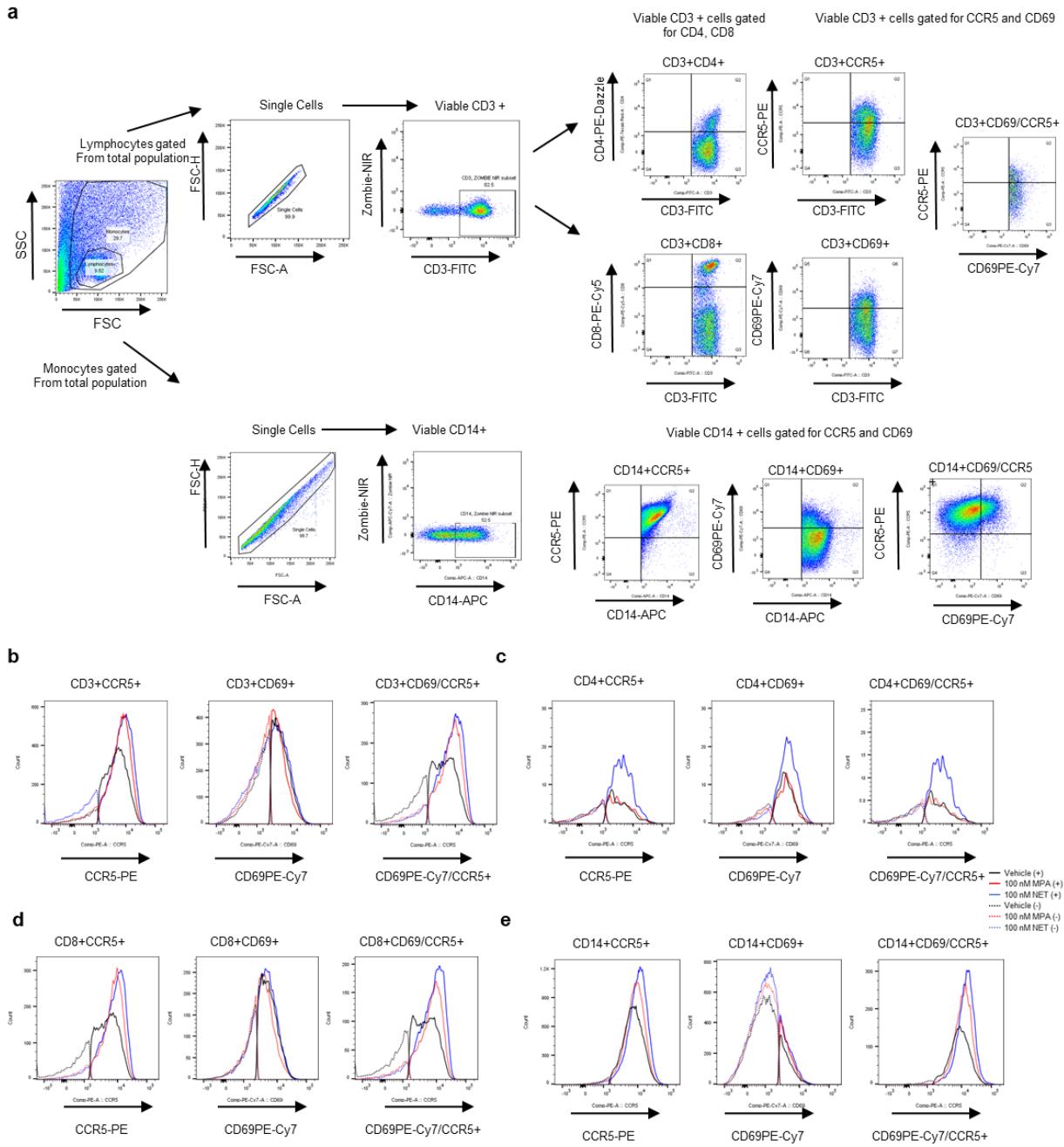


Figure S6. Representative gating strategy for analysis of viable lymphocytes and monocytes isolated from ectocervical explants 48 hr and 7 days post treatment.

Ectocervical explants were treated in parallel with 100 nM MPA or NET or a vehicle control (EtOH) for 48 hr or 7 days followed by tissue digestion. Thereafter cells were stained for different cell surface receptors and analysed by flow cytometry. Graphs show the gating

strategy of one representative donor at day 7 post treatment. (a) Monocytes and lymphocytes were gated from the total cellular population, after which only the single cell population was used for analysis. Viable CD3+ cells were gated using the ZOMBIE NIR viability stain and CD3 marker. For lymphocytes, CD4 and CD8 cells were gated from the viable CD3+ population and CCR5 and CD69 cells using the appropriate MFO controls. For monocytes, viable CD14+ cells were used and CCR5+ and CD69+ expression levels were determined using the appropriate MFO controls. (b-e) Representative histogram distributions of CCR5+, CD69+ or CD69+ CCR5+ expressing (b) CD3+ cells, (c) CD4+ cells, (d) CD8+ cells, (e) CD14+ cells following 7-day incubation with ligands. Solid line indicates + population, dashed line indicates negative population based on MFO.

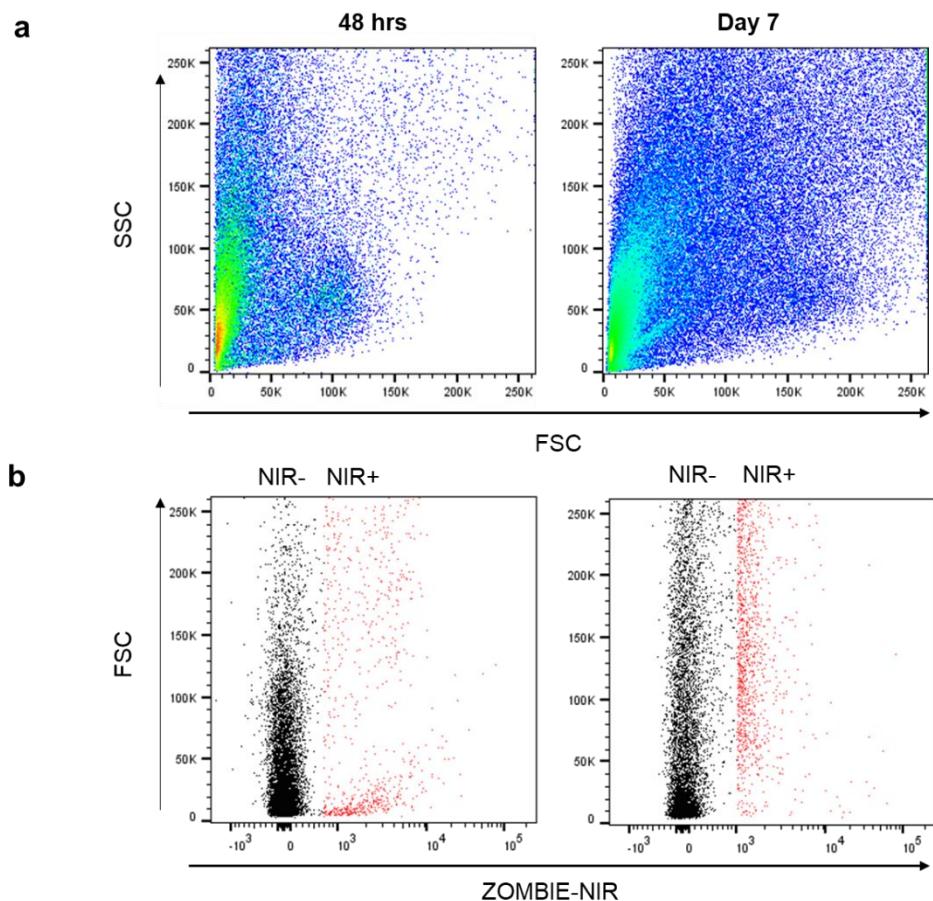


Figure S7. Lymphocytes acquired after a tissue digestion of ectocervical explants are viable post treatment.

Ectocervical explant tissue were stimulated in parallel with vehicle (EtOH) or 100 nM MPA or 100 nM NET for 48 hr or 7 days after which tissue was digested and processed for flow cytometry analysis. (a) Representative ungated FSC vs SSC donor dot plots after 48 hr and 7 days are shown. (b) Viability analysis done on ungated populations to assess the % viability of cells. Representative donor dot plots showing viability analysis 48 hr and Day 7 post vehicle stimulation on ungated populations using the ZOMBIE NIR viability stain. The % viability of ungated population was calculated and indicated in supplementary Table S7.

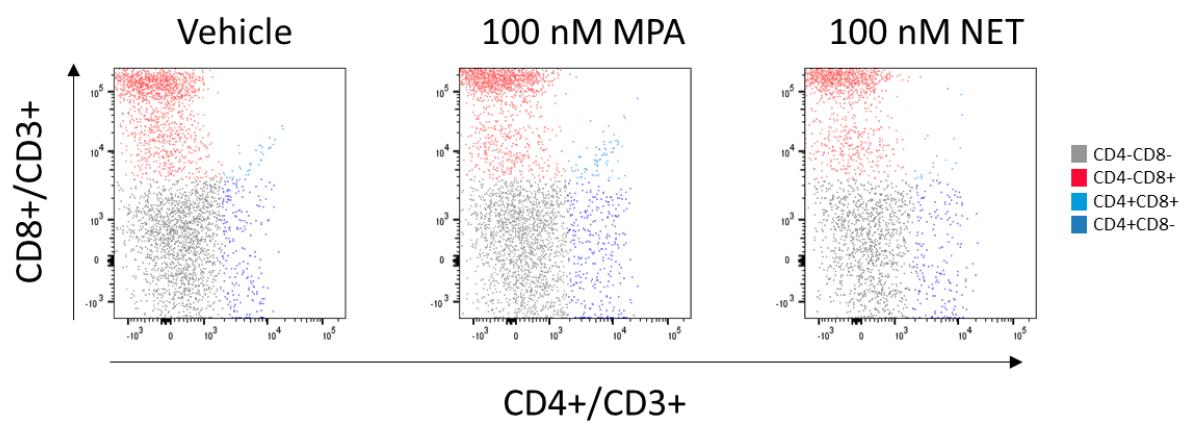


Figure S8. Representative scatter plots of CD8 and CD4 scatter plot of CD3+ cells of the 3 treatment groups at day 7.

Ectocervical explant tissue were stimulated in parallel with vehicle (EtOH) or 100 nM MPA or 100 nM NET for 7 days after which the frequency of CD4+ and CD8+ of live CD3+ cells were determined using flow cytometry.